Prognostic Value of Plasma von Willebrand Factor and Soluble P-Selectin as Indices of Endothelial Damage and Platelet Activation in 994 Patients With Nonvalvular Atrial Fibrillation

Dwayne S.G. Conway, MRCP; Lesly A. Pearce, MS; Bernard S.P. Chin, MRCP; Robert G. Hart, MD; Gregory Y.H. Lip, MD

Background—Abnormal plasma markers of a prothrombotic state have been described in atrial fibrillation (AF), but no such marker has yet been shown to reliably predict future stroke or cardiovascular outcome in AF. We hypothesized that raised plasma levels of von Willebrand factor (vWF, an index of endothelial damage/dysfunction) and/or soluble P-selectin (sP-sel, an index of platelet activation) might predict vascular events in AF.

Methods and Results—We measured vWF and sP-sel levels by ELISA in 994 participants receiving aspirin in the Stroke Prevention in Atrial Fibrillation III trial, at study entry or after 3 months, and related these indices to the subsequent incidence of stroke and vascular events. Plasma vWF levels were a significant predictor of both stroke ($P<0.03$) and vascular events ($P<0.001$), with the greatest risk for those with the highest levels of vWF. After adjustment for other clinical predictors, the relationship between vWF and stroke became nonsignificant, but vWF remained an independent predictor of vascular events (relative risk, 1.2 [95% CI, 1.0–1.4] per 20 IU/dL increase in vWF; $P=0.02$). No significant relationships were found between sP-sel levels and outcome.

Conclusion—Among patients with AF who received aspirin, raised levels of vWF (endothelial damage/dysfunction) were predictive of stroke and vascular events, but raised sP-sel levels (platelet activation) were not associated with increased cardiovascular risk. Endothelial damage/dysfunction (or vWF itself) may play an important role in the mechanisms behind stroke and cardiovascular outcome among aspirin-treated AF patients and might represent a target for novel therapies or an adjunctive aid to risk stratification in AF. (Circulation. 2003;107:3141-3145.)

Key Words: von Willebrand factor • stroke • prognosis • atrial fibrillation

Nonvalvular atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and is associated with a $\approx$5-fold increase in stroke risk,1 but several additional clinical and echocardiographic features further influence the overall risk of stroke in AF.2,3 Abnormal plasma markers of coagulation, endothelial function, and platelet activation have been described in AF,4–8 some of which may predict thrombus or prothrombotic features visible in the left atrium or left atrial appendage (LAA) by transesophageal echocardiography (TEE).9–11 However, no plasma marker has yet been shown to reliably predict stroke or cardiovascular mortality and morbidity in AF. The identification of such a plasma marker(s) could be of clinical value, perhaps in refining the process of thromboembolic risk stratification in AF, and might possibly represent a target for the development of future antithrombotic therapies.

We recently demonstrated independent associations between several epidemiological stroke risk factors and elevated plasma levels of von Willebrand factor (vWF, a marker of endothelial damage/dysfunction12) among a large cohort of patients with AF,13 with a significant stepwise increase in mean vWF levels between those judged to be at low, moderate, and high risk of stroke using a prospectively validated risk stratification scheme. In the same cohort, we found elevated plasma levels of soluble P-selectin (sP-sel, a marker of platelet activation12) to be related to risk factors for atherosclerosis but unrelated to estimated stroke risk.13 To determine whether raised plasma levels of vWF and sP-sel might independently predict stroke or the composite end point of stroke, myocardial infarction (MI), or vascular death in AF, we measured baseline plasma levels of vWF and sP-sel in 994 participants receiving aspirin 325 mg/day (alone or
combined with fixed inefficacious doses of warfarin" in the Stroke Prevention in Atrial Fibrillation (SPAF) III study and related these indices to the risk of subsequent stroke and vascular events.

**Methods**

The design and main results of the SPAF III study have been reported previously. In brief, patients with nonvalvular AF were stratified as having low, moderate, or high risk for stroke on the basis of clinical and echocardiographic features predictive of thromboembolic risk in the earlier SPAF I and II studies. Those with any of 4 high-risk criteria (women >75 years of age, systolic hypertension >160 mm Hg, impaired left ventricular function, or previous thromboembolism) were randomized to receive either adjusted-dose warfarin (target international normalized ratio [INR] 2 to 3) or fixed, low-dose warfarin (target INR 1.2 to 1.5) plus aspirin 325 mg/day (termed combination therapy). Participants without any of the 4 specific risk factors were classified as being at moderate or low risk (depending on the presence or absence of hypertension) and received aspirin 325 mg/day alone.

Blood samples were primarily collected at baseline (or after 3 months of enrollment if not available at baseline) from all participants except those enrolled and followed up at outlying clinics where specimens could not be adequately processed; thus, 69% (1339/1936) of SPAF III participants had 1 sample collected at baseline or at 3 months. To remove the potentially confounding effect of vascular risk in the earlier SPAF I and II studies. Those with any of 4 high-risk criteria (women >75 years of age, systolic hypertension >160 mm Hg, impaired left ventricular function, or previous thromboembolism) were randomized to receive either adjusted-dose warfarin (target international normalized ratio [INR] 2 to 3) or fixed, low-dose warfarin (target INR 1.2 to 1.5) plus aspirin 325 mg/day (termed combination therapy). Participants without any of the 4 specific risk factors were classified as being at moderate or low risk (depending on the presence or absence of hypertension) and received aspirin 325 mg/day alone.

**Blood Collection and Laboratory Analysis**

Blood collection materials were prepared at the Laboratory for Clinical Biochemistry Research, Department of Pathology, University of Vermont (Burlington, Vt). Blood for vWF and sP-sel assays was drawn into 3.8% sodium citrate tubes (Becton Dickinson), immediately mixed by gentle inversion, stored on melting ice, and centrifuged at 4°C for 30 000g minutes within 1 hour of phlebotomy, and then plasma was separated for vWF and sP-sel assays. Measurements of sP-sel and vWF were performed using ELISA with reagents from R&D Systems (Abington, UK) and Dako-Patts (Ely, UK), respectively. The unit for vWF is IU/dL and was standardized by reference vWf from the National Institute for Biological Standards and Controls (Potters Bar, Hertfordshire, UK). Intra-assay coefficients of variation for all ELISA assays were <5%, and interassay variances were <10%.

**Data Analysis**

Plasma vWF and sP-sel were analyzed both as continuous variables and after division into tertiles, and their relationships to two end points, ischemic stroke and vascular events, during 2 years of follow-up were assessed. A vascular event was defined as the first occurrence of ischemic stroke, MI, or vascular death. Survival curves were computed using the Kaplan-Meier method and compared between groups using the log-rank test. Unadjusted and adjusted relative risks (RRs) (and their 95% confidence intervals) were estimated using Cox proportional hazard ratios with statistical significance determined by the likelihood ratio test. Multivariate analyses adjusting for patient characteristics previously identified as predictive of stroke were determined to be independent contributions of plasma vWF and sP-sel to stroke risk. Similar analyses were done for the vascular end point with adjustment for other risk factors for vascular event first being identified through fitting of forward and backward stepwise Cox models. All tests were two sided, and statistical significance was accepted at the 0.05 level. Statistical analyses were undertaken using SPSS software.

**Results**

Characteristics of our 994 patients at entry were similar to those of all SPAF III participants assigned aspirin or combination therapy (Table) with 69% of our cohort being low- to moderate-risk patients by SPAF criteria. Mean values (±SD) for vWF and sP-sel were 145 ± 31 IU/dL and 34 ± 13 ng/mL, respectively, and cut points for tertiles were 131 and 158 IU/dL for vWF and 28 and 38 ng/mL for sP-sel. During the follow-up period, stroke occurred in 39 patients (3.0%/per patient-year), and a vascular event occurred in 68 (5.3%/per patient-year).

**von Willebrand Factor**

A higher rate of stroke occurred with higher tertiles of vWF (Figure 1A, P=0.03; relative risk [RR] 1.9, 95% CI 0.8–4.9 [middle versus lowest tertile]; RR 3.0, 95% CI 1.2–7.1 [upper versus lowest tertile]). The relationship was also significant with vWF as a linear variable (RR 1.3 per increase of 20 IU/dL, 95% CI 1.0–1.6; P=0.02). After adjusting for age, elevated systolic blood pressure, and prior cerebral ischemia, statistical significance was lost in the relationship between tertile of vWF and stroke (RR 1.7, 95% CI 0.7–4.3 [middle versus lowest tertile]; RR 2.3, 95% CI 1.0–5.6 [upper versus lowest]; P=0.13), and the linear relationship between vWF and stroke risk was of marginal statistical significance (RR 1.2 per increase of 20 IU/dL, 95% CI 1.0–1.5; P=0.06).

Rates of vascular events were significantly higher with higher tertiles of vWF (Figure 1B, P<0.001). After adjusting for age, prior cerebral ischemia, hypertension, diabetes, and moderate to severe left ventricular dysfunction, level of vWF...
by tertile (RR 1.6, 95% CI 0.8–3.3 [middle versus lowest tertile]; RR 2.5, 95% CI 1.2–5.0 [upper versus lowest tertile]; \( P = 0.02 \)) or as a continuous measure (RR 1.2 per increase of 20 IU/dL, 95% CI 1.0–1.4; \( P = 0.02 \)) remained a significant predictor of subsequent vascular events.

Soluble P-Selectin
Whether analyzed by tertiles (Figure 2A, \( P = 0.6 \)) or as a continuous variable (RR 0.7 per increase of 20 ng/mL, 95% CI 0.4–1.2; \( P = 0.2 \)), no relationship was seen between sP-sel and stroke. Similarly, no relationship was seen between sP-sel and vascular events (Figure 2B, \( P = 0.4 \)). Adjustment for clinical predictors did not reveal any significant relationship between sP-sel, either by tertile or continuously, and either end point (all \( P > 0.2 \)).

Cardioembolic Versus Noncardioembolic Stroke
Of the 39 first strokes that occurred during follow-up, 20 were classified as cardioembolic and 10 as noncardioembolic with the remaining 9 indeterminate primarily as a result of a lack of proximate imaging of the carotid arteries.17 Patients in the lowest tertile of vWf only suffered cardioembolic strokes, whereas those strokes occurring in patients in the middle and highest tertiles of vWf were ≈60% cardioembolic and 40% noncardioembolic strokes (\( P = 0.05 \)).

Discussion
In this longitudinal study of a cohort of 994 participants with AF receiving aspirin 325 mg/day for thromboprophylaxis as part of the SPAF III study, baseline plasma levels of vWf were univariately predictive of subsequent stroke and vascular events. After adjusting for other vascular risk factors, vWf remained independently predictive of vascular events.

Although the mechanism(s) behind stroke and thromboembolism in AF is incompletely understood,18 the increased risk is mainly due to the embolization of thrombus initially formed within the LAA. More than 150 years ago, Virchow suggested that the induction of thrombogenesis requires a triad of abnormal factors (vessel wall, blood flow, and intravascular procoagulant factors); the application of Virchow’s triad to AF now seems appropriate. Reduced LAA blood flow velocity, dense spontaneous echocontrast (an index of left atrial stasis), and complex aortic plaque seen on
TEE have each been shown to independently predict LAA thrombus and stroke in AF,\(^\text{19-21}\) reflecting the importance of both localized blood pool stasis and generalized atheromatous (or “vessel wall”) disease in the etiology of LAA thrombogenesis. Furthermore, abnormally high circulating plasma markers of coagulation, endothelial function (including vWF), and platelet activation (including sP-sel) have been previously described in AF compared with healthy controls.\(^\text{4-8,22}\) and raised levels of some of these plasma markers have been shown to be associated with the presence of thrombus or prothrombotic features visible within the LAA by TEE.\(^\text{9-11}\) However, this study is the first to demonstrate that raised levels of one such marker (vWF) can predict clinical outcome (including stroke) in AF, although uncertainty remains as to whether it is AF itself or simply the presence of additional underlying cardiovascular conditions that determines elevation of these markers in AF.\(^\text{13,22,23}\)

Because of the exclusion of patients assigned adjusted-dose warfarin, our cohort featured predominantly low- to moderate-risk patients (thus, the number of incident strokes were relatively few), and all were treated with aspirin, factors that must be considered before applying our study findings to all populations with AF. Nonetheless, it is notable that although plasma levels of vWF (an index of endothelial damage/dysfunction) predicted stroke and the composite end point of stroke, MI, or vascular death among our whole cohort of 994 AF patients, plasma levels of sP-sel (an index of platelet activation) did not, and in a previous substudy from the SPAF III trial, 4 other prothrombotic markers (fibrinogen, β-thromboglobulin, prothrombin fragment F1.2, and factor V Leiden) also failed to predict outcome.\(^\text{15}\) Thus, endothelial damage/dysfunction, or vWF itself, may be of particular importance in the prothrombotic state of AF. We recently demonstrated, among an expanded cohort of AF patients from the SPAF III study (including the 994 individuals from our current analysis plus a greater proportion deemed at high risk of stroke as a result of the inclusion of 327 patients randomized to receive warfarin therapy), that plasma vWF (but not sP-sel) levels significantly increased in line with increased stroke risk as estimated using validated risk-stratification criteria.\(^\text{15}\) Indeed, elevated plasma vWF levels were independently associated with several epidemiological risk factors for stroke in AF, whereas plasma sP-sel levels were mainly elevated in association with risk factors for atherosclerotic disease.\(^\text{13}\)

A dominant role of the endothelium and a lesser role for platelets might explain the relatively disappointing efficacy of antplatelet therapy in prevention of stroke in AF.\(^\text{16}\) However, it is also theoretically possible that the use of aspirin as thromboprophylactic therapy in the present study might have negated platelet activation (as assessed by sP-sel) as a potential mechanism of thromboembolism, and we therefore cannot exclude a role for platelets in stroke among AF patients not on antplatelet therapy.

Current clinical practice for prevention of thromboembolic stroke in AF is limited not only by the disappointing efficacy of antplatelet therapy\(^\text{16}\) but also by the hemorrhagic complications and the need for INR monitoring inherent with warfarin therapy.\(^\text{24}\) The finding that, in addition to a relationship between vWF and stroke risk factors,\(^\text{13}\) plasma vWF may prospectively predict stroke and (independently) vascular events might have implications for the future of cardiovascular risk assessment in AF. Furthermore, vWF (or the vascular endothelium) may present a target for the development of novel thromboprophylactic agents in AF.

In this study, we note that a greater proportion of strokes among those with higher levels of vWF are likely to be noncardioembolic in origin (of borderline significance, \(P=0.05\)); thus, it may be that the observed relationship between vWF and stroke/vascular events in AF relates to widespread endothelial damage/dysfunction and local atherothrombosis rather than to thromboembolism from the LAA. However, we are cautious in our interpretation of this subgroup analysis because our study consisted mainly of patients with low to moderate risk of stroke (or thromboembolic risk), and a body of evidence already exists suggesting that vWF may indeed be linked to LAA thrombosis in AF.\(^\text{10,25}\) Certainly, Heppel et al\(^\text{10}\) found raised plasma levels of vWF to be predictive of the presence of LAA thrombus visible by TEE, whereas Fukuchi et al\(^\text{25}\) found a significant correlation between the degree of endocardial expression of vWF and the degree of platelet adhesion/thrombus formation in the atrial appendage. Furthermore, Goldsmith et al\(^\text{26}\) found raised plasma vWF associated with damaged atrial appendage endocardium in patients with mitral valve disease and (in most cases) AF. Further studies are therefore needed to establish the true mechanism of stroke associated with raised plasma vWF in AF, to evaluate the endothelium/endocardium (or vWF itself) as a target for novel antithrombotic therapies in AF, to investigate other indices of endothelial dysfunction in AF, and to examine the potential for plasma vWF as an aid to clinical risk stratification in AF.

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References

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